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(54) Polymorphisms in the human organic anion transporter C (OATP-C) gene

(57) This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic var-

iations in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs such as statins.

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Description

- [0001] This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs.
- [0002] Na⁺-independent organic anion transporting polypeptide (OATP) C gene is a member of the OATP supergene family involved in multifunctional transport of organic anion. OATPC transports the organic anion taurocholate, conjugated steroids: DHEAS, estradiol 17 β -D-glucuronide and estrone-3-sulfate, eicosanoids: PGE₂, thromboxane B₂, leukotriene C₄, and E₄, and thyroid hormones T4 and T3 1,2. OATPC has also been shown to be involved in the transport of xenobiotics, and drugs involved in lipid lowering e.g. statins¹. Statins have been referred to as a first-line therapy for patients with atherosclerotic vascular diseases. The OATPC gene and its product is also thought to be of importance in other diseases due to its transport of DHEAS an adrenal steroid which has been suggested to have positive neuropsychiatric, immune, and metabolic effects³. Due to the substrate specificity, location in the liver, and being exclusively expressed in the liver, Abe *et al* suggested that OATPC could be the predominant clearance mechanism of several endogenous and exogenous substrates in the liver. OATPC is the first human molecule reported to transport thyroid hormones².
- [0003] This liver specific transporter may be useful in liver-specific drug delivery systems and liver-specific chemotherapy, bile acid formation and the pathogenesis of diseases such as cholestasis, hyperbilirubinemia and thyroid hormone resistance.
- [0004] The OATPC gene (sometimes called OAPT2 in the literature) has been cloned by four different groups, annotated and published as EMBL accession numbers AB026257 (OATPC, 2452bp), AF205071(OATP2, 2830, ref 1), AJ132573(OATP2, 2778)⁴, and AF060500 (LST-1)². Polymorphism has been reported by Tamai⁵ which is Asn130Asp and Val174Ala although any functional effect was stated therein to be not clear. Konig (2000) J Biol Chem 275, 23161-23168 describes the genomic organisation of OATP 1, 2 and 8. International patent application WO 00/08157 describes human anion transporter genes and some polymorphisms.
- [0005] All positions herein of polymorphisms in the OATPC polynucleotide relate to the position in one of SEQ ID NO 1 or 3-12 unless stated otherwise or apparent from the context.
- [0006] All positions herein of polymorphisms in the OATPC polypeptide relate to the position in SEQ ID NO 2 unless stated otherwise or apparent from the context.
- [0007] One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, 43, 254; Marshall (1997), Nature Biotechnology, 15, 1249; International Patent Application WO 97/40462, Spectra Bio-medical; and Schafer *et al.* (1998), Nature Biotechnology, 16, 33.
- [0008] Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.
- [0009] Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature), position, new amino acid. For (a hypothetical) example "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.
- [0010] The present invention is based on the discovery of polymorphisms in OATPC.
- [0011] According to one aspect of the present invention there is provided a method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one polymorphic position and determining the status of the human by reference to polymorphism in the OATPC gene. Preferred polymorphic positions are one or more of the following positions:

A method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

positions 510, 696, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined

¹ A Novel Human Hepatic Organic Anion Transporting Polypeptide (OATP2), Hsiang *et al* J Biol Chem 274, 37161-37168 (1999)

² Identification of a Novel Gene Family Encoding Human Liver-specific Organic Anion Transporter LST-1, Takaaki Abe *et al* J Biol Chem 274, 17159-17163 (1999)

³ Bates *et al* (1998) Curr. Opin. Endocrinol. Diab. 5, 357-366

⁴ A novel human organic anion transporting polypeptide localised to the basolateral hepatocyte membrane, Konig Jorg *et al* (2000) Am J Physiol. Gastrointest. Liver Physiol. 278: G156-G164

⁵ Tamai *et al* (2000), BBRC, 273, 251-60

by the position in SEQ ID NO: 1;
 positions 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
 positions 321 and 1332 defined by position in SEQ ID NO 3;
 5 position 41 defined by position in SEQ ID NO 4;
 positions 109 and 244 defined by position in SEQ ID NO 5;
 positions 117 and 283 defined by position in SEQ ID NO 6;
 positions 209 and 211 defined by position in SEQ ID NO 7;
 positions 63 to 68 defined by position in SEQ ID NO 8;
 10 position 53 defined by position in SEQ ID NO 9;
 position 75 defined by position in SEQ ID NO 10;
 position 162 defined by position in SEQ ID NO 11; and
 positions 84 defined by position in SEQ ID NO 12.

- 15 [0012] The term human includes both a human having or suspected of having a OATPC mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.
- [0013] The term polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene.
- 20 [0014] The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.
- [0015] The status of the individual may be determined by reference to allelic variation at any one, two, three, four, five, six, seven, eight, nine or more positions.
- [0016] The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.
- 25 [0017] It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection 30 of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. 43, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.
- 35

Abbreviations:	
ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay

(continued)

Abbreviations:	
5	NASBA Nucleic acid sequence based amplification
	OATP Na+-independent organic anion transporting polypeptide
	OLA Oligonucleotide ligation assay
10	PCR Polymerase chain reaction
	PTT Protein truncation test
	RFLP Restriction fragment length polymorphism
	SDA Strand displacement amplification
15	SNP Single nucleotide polymorphism
	SSCP Single-strand conformation polymorphism analysis
	SSR Self sustained replication
20	TGGE Temperature gradient gel electrophoresis

Table 1 -

Mutation Detection Techniques**General:** DNA sequencing, Sequencing by hybridisation**Scanning:** PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage**Hybridisation Based**

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, 14, 303; WO 95/13399 (Public Health Inst., New York)**Extension Based:** ARMSTM, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, 17, 2347.**Incorporation Based:** Mini-sequencing, APEX**Restriction Enzyme Based:** RFLP, Restriction site generating PCR**Ligation Based:** OLA**Other:** Invader assay

* Note: not useful for detection of promoter polymorphisms.

Table 2 -

Signal Generation or Detection Systems**Fluorescence:** FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)**Other:** Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 -

Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

[0018] Preferred mutation detection techniques include ARMSTM, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

[0019] Particularly preferred methods include ARMSTM and RFLP based methods. ARMSTM is an especially preferred method.

[0020] In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of a drug transportable by OATPC.

- [0021] Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.
- [0022] Individuals who carry particular allelic variants of the OATPC gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.
- [0023] In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by OATPC. This may be particularly relevant in the development of hyperlipoproteinemia and cardiovascular disease and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.
- [0024] In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the OATPC gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.
- [0025] In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.
- [0026] According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a variant allelic polymorphism at one or more of positions defined herein or a fragment thereof of at least 20 bases comprising at least one novel polymorphism.
- [0027] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.
- [0028] According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human OATPC gene and comprising an allelic variant selected from any one of the following:

	Region	variant	Position in SEQ ID NO	SEQ ID NO
25	Exon 4	A	510	1
30	Exon 5	T	670	1
35	Exon 5	T	696	1
40	Exon 9	G	1299	1
45	Exon 9	A	1312	1
50	Exon 9	A	1347	1
55	Exon 10	C	1561	1
	Exon 14	C	2028	1
	3'UTR	Insert T	2327	1
	3'UTR	C	2342	1
	Promoter	G	321	3
	Promoter	C	1332	3
	Intron 1	A	41	4
	Intron 2	G	109	5
	Intron 2	G	244	5
	Intron 3	A	117	6
	Intron 3	A	283	6
	Intron 4	A	209	7
	Intron 4	A	211	7
	Intron 4	Deletion CTTGTA	63	8
	Intron 6	T	53	9

(continued)

Region	variant	Position in SEQ ID NO	SEQ ID NO
Intron 9	Insert TTC	75	10
Intron 11	Insert T	162	11
Intron 12	C	84	12

- 10 [0029] According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a polymorphism, preferably corresponding with one or more of the positions defined herein or a fragment thereof of at least 20 bases comprising at least one polymorphism.
- [0030] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.
- [0031] The invention further provides a nucleotide primer which can detect a polymorphism of the invention.
- 15 [0032] According to another aspect of the present invention there is provided an allele specific primer capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions as defined herein.
- [0033] An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.
- 20 [0034] An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.
- 25 [0035] Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.
- [0036] According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions defined herein.
- 30 [0037] The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.
- [0038] The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.
- 35 [0039] According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a OATPC gene polymorphism at one of the positions defined herein.
- [0040] According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.
- 40 [0041] The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.
- 45 [0042] In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphisms of relatively high frequency. The OATPC gene is on chromosome 12p (as shown from a database search with the cDNA as a query sequence). Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation

at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) 62:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) 93:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D.

[0043] According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis.

[0044] According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug transportable by OATPC in which the method comprises:

- i) detection of a polymorphism in OATPC in the human, which detection comprises determining the sequence of the human at one or more of the following positions:

15 positions 487, 510, 554, 670, 696, 819, 820, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
 positions 130, 152, 174, 241, 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
 positions 321 and 1332 defined by position in SEQ ID NO 3;
 position 41 defined by position in SEQ ID NO 4;
 20 positions 109 and 244 defined by position in SEQ ID NO 5;
 positions 117 and 283 defined by position in SEQ ID NO 6;
 positions 209 and 211 defined by position in SEQ ID NO 7;
 positions 63 to 68 defined by position in SEQ ID NO 8;
 position 53 defined by position in SEQ ID NO 9;
 25 position 75 defined by position in SEQ ID NO 10;
 position 162 defined by position in SEQ ID NO 11; and
 positions 84 defined by position in SEQ ID NO 12.

and determining the status of the human by reference to polymorphism in the OATPC gene; and
 30 ii) administering an effective amount of the drug.

[0045] Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which statin drug or drugs to administer and/or in deciding on the effective amount of the statin drug or drugs. Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin.
 35 The reader is referred to the following references for further information: Drugs and Therapy Perspectives (12th May 1997), 9: 1-6; Chong (1997) Pharmacotherapy 17: 1157-1177; Kellick (1997) Formulary 32: 352; Kathawala (1991) Medicinal Research Reviews, 11: 121-146; Jahng (1995) Drugs of the Future 20: 387-404, and Current Opinion in Lipidology, (1997), 8, 362 - 368. A preferred statin drug is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry 5: 437-444; now called rosuvastatin, see Olsson (2001) American Journal of Cardiology, 87, supplement 1, 33-36. The term "drug transportable by OATPC" means that transport by OATPC in humans is an important part of a drug exerting its pharmaceutical effect in man. For example, some statins have to be transported to the liver by OATPC to exert their lipid lowering effects.

[0046] According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug transportable by OATPC in which the method comprises:

45 i) diagnosis of a single nucleotide polymorphism in OATPC gene in the human, which diagnosis preferably comprises determining the sequence of the nucleic acid at position 487 in the coding sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
 and determining the status of the human by reference to polymorphism in the OATPC gene; and
 50 ii) administering an effective amount of the

[0047] Although the polymorphism at position 487 (A→G; Asn130Asp) was reported by Tarnai, no functional effect was attributed. Indeed the following polymorphisms are known but have not previously been attributed a role in statin pharmacogenetics as disclosed herein.

55

OATPC polymorphisms
Position of nucleotide as in sequence 1 of OATPC patent

(continued)

OATPC polymorphisms						
Position of amino acid as in sequence 2 of OATPC patent						
Exon	nucleotide	SNP	Aminoacid	Change	Change	
4	487	A>G	130	Asn>Asp	N>D	
4	554	G>A	152	Arg>Lys	R>K	
5	620	T>C	174	Val>Ala	V>A	
5	670	C>T	191	Leu	L	
6	819	A>T	240	Val	V	
6	820	G>A	241	Asp>Asn	D>N	

- 15 [0048] According to another aspect of the present invention there is provided use of a drug transportable by OATPC in preparation of a medicament for treating a disease in a human diagnosed as having a polymorphism therein, preferably at one or more of the positions defined herein. Preferably the disease is cardiovascular.
- 10 [0049] According to another aspect of the present invention there is provided a pharmaceutical pack comprising OATPC transportable drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism therein, preferably at one or more of the positions defined herein.
- 20 [0050] According to another aspect of the present invention there is provided an allelic variant of human OATPC polypeptide comprising at least one of the following:
- 25 a leucine at position 400 of SEQ ID NO 2;
 an isoleucine at position 405 of SEQ ID NO 2;
 an arginine at position 488 of SEQ ID NO 2;
 a phenylalanine at position 643 of SEQ ID NO 2;
- 30 or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic variant.
- [0051] Fragments of polypeptide are at least 10 amino acids, more preferably at least 15 amino acids, more preferably at least 20 amino acids.
- 35 [0052] According to another aspect of the present invention there is provided an antibody specific for an allelic variant of human OATPC polypeptide as described herein.
- [0053] Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example F(ab)₂, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the allelic variant of OATPC with a K_a of greater than or equal to about 10⁷ M⁻¹. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N. Y. Acad. Sci.*, 51:660(1949).
- 40 [0054] Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.
- 45 [0055] Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).
- 50 [0056] The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

[0057] Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

5 [0058] According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

[0059] The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

[0060] In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

10 [0061] AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

[0062] General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

[0063] Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

15 **Example 1**

Identification of Polymorphisms

1. Methods

20 **DNA Preparation**

25 [0064] DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

30 [0065] Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR. Where described below, the primary fragment was diluted 1/100 and two microlitres were used as template for amplification of secondary fragments. PCR was performed in two stages (primary fragment then secondary fragment) to ensure specific amplification of the desired target sequence.

Polymorphisms in OATPC: cDNA screening of 15 Liver samples					
	Region	SNP	Position	Amino Acid Change	Allele frequencies
40	Exon 4	G/A	510	None	G=96.7% A=3.3%
	Exon 5	C/T	670	None	C=50% T=50%
45	Exon 5	C/T	696	None	C=60% T=40%
	Exon 9	C/G	1299	Phe400Leu	C=96.7% G=3.3%
50	Exon 9	G/A	1312	Va1405Ile	G=96.7% A=3.3%
	Exon 9	G/A	1347	None	G=96.7% A=3.3%
55	Exon 10	G/C	1561	Gly488Arg	G=96.7% C=3.3%

(continued)

Polymorphisms in OATPC: cDNA screening of 15 Liver samples				
Region	SNP	Position	Amino Acid Change	Allele frequencies
Exon 14	A/C	2028	Leu643Phe	A=90% C=10%

[0066] OATP2 above refers to the clone sequenced by Hsiang et al (ref 1). Some comment on the numbering of exons in OATPC is required. This gene contains an exon (38 bp) upstream (5' UTR region) of the exon containing the ATG start site for translation. Therefore the exon numbering could vary depending whether this exon is counted as the first exon or not. In the literature, Konig (2000) JBC 275: 23161-68, have defined exon 1 as that containing the ATG start site and therefore we have adopted the same numbering in this application (but note that the priority document relating to the present application did vice versa; for example, exon 5 in this application is equivalent to exon 6 in the priority document).

PCR PRODUCTS				
Fragment	Forward Oligo	Reverse Oligo		
443-999	443-466	979-999		
874-1360	874-896	1337-1360		
1255-1684	1255-1278	1663-1684		
1559-2095	1559-1581	2073-2095		

RFLP analysis				
Polymorphism	Position	RFLP Enzyme/PCR size	RFLP fragment size	
G/A	510			
C/T	670	BmR I/595bp	C=349bp, 246bp T=595bp	
C/T	696			
C/G	1299	Apo I/ 595bp	C=30bp, 60bp, 380bp G=90bp, 380bp	
G/A	1312	Bst 4CI/ 595bp	G=77bp, 393bp. A=470bp	
G/A	1347			
G/C	1561	HpyCH4IV/595bp	G=470bp C=144bp, 326bp	
A/C	2028	Ase I/ 577bp	A=89bp, 488bp C=577bp	

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Example 2

{0067}

Further OATPC Polymorphisms							
SNPs in OATPC 3'UTR (positions according to SEQ ID NO 1)							
	Exon	Nucleotide	SNP			Frequency	Frequency
				Caucasian	Japanese		
10	3' UTR	2327	Ins T			not screened	0.1
15	3' UTR	2342	T>C			not screened	0.4
SNPs in OATPC promoter (positions according to SEQ ID NO 3)							
		Nucleotide	SNP			Frequency	Frequency
				Caucasian	Japanese		
20		321	T>G			0.03	not screened
25		1332	A>C			0.08	not screened
SNPs in OATPC introns							
	Intron	Nucleotide	SNP	Nucleotide	Sequence		
						position	ID No
		relation to exon		in sequence			
30	1	IVS1+21	T>A	41	4		
35	2	IVS2+89	T>G	109	5		
40	2	IVS2+224	A>G	244	5		
45	3	IVS3+97	C>A	117	6		
50	3	IVS3+263	G>A	283	6		
55	4	IVS4+189	G>A	209	7		
	4	IVS4+191	G>A	211	7		
	4	IVS5-118	delCTTGTA	63	8		
	6	IVS6+33	C>T	53	9		
	9	IVS10-107	ins TTC	75	10		
	11	IVS11+142	Ins T	162	11		
	12	IVS13-97	G>C	84	12		

OATPC intronic SNPsKey

5 [0068] 20bp of exon sequence shown in uppercase
 Intron sequence in lowercase (200 to 300bp only)
 SNP shown in uppercase (one allele only)

10 Sequence ID No 4
 IVS1+21 T>A SNP at position 41 in this sequence
 GATACTGC~~A~~ TGGATTGAAG gtagaataag ttttatgtt Ttgagctaaa ataagtaat 60
 agggaaacttt aatgtataga aaagcaagg ttaaaaaaga acattatgtt tc~~a~~attata 120
 15 atttcaatt gaagcatata ttgaaatatt aacataatga ttcatacctt gat~~t~~taaacc 180
 agtctttaa tctgattaag 200

20 Sequence ID No 5
 IVS2+89 T>G SNP at position 109 in this sequence
 IVS2+224 A>G SNP at position 244 in this sequence
 TGACGGAAGC TTTGAAATTG gtaacattt ttttctattt taataacca acttgcaaag 60
 ttaaaaaata tatatgc~~t~~ttt acaccaactgg ttatcaactg gg~~t~~taatTt atctctcaca 120
 25 ggcaatttgg caataactaa aaacatttg~~t~~ ggttgtcata actgcacagg gg~~t~~ggggc 180
 aat~~g~~gaagt~~g~~tg ctactggat c~~t~~aaaggtag aggtcagg~~g~~gg tactgctaaa tattctataa 240
 tg~~c~~A~~c~~aaaga at~~g~~at~~g~~taac tgaaaatgtt gat~~g~~tgagg at~~g~~ttcagaa accctgattc 300

30 Sequence ID No 6
 IVS3+97 C>A SNP at position 117 in this sequence
 IVS3+263 G>A SNP at position 283 in this sequence

35 CCACATTTCT TCATGGGATA gtaagtgtt aaaaaaaaaa aaacctctgt gccactatca 60
 gtac~~c~~ttgt~~a~~ aatttaggagt agaa~~t~~ttt~~t~~ tattatcc~~c~~ ttaat~~a~~aggc agttac~~C~~ttt 120
 40 tgagaagata cccactaagt gtgtacagaa at~~g~~aaat~~g~~tg~~t~~ gtctattt~~t~~ ctacataatc 180
 attttattt~~a~~ tc~~t~~gtagctt catatacttt gaaataacaa aaagactaaa ctgtagagg~~t~~ 240
 tcaa~~a~~atgaaa taaataggct ttttat~~g~~aat tt~~t~~tagtatac acGtata~~tac~~ t~~g~~tacgtctt 300

45 Sequence ID No 7
 IVS4+189 G>A SNP at position 209 in this sequence
 IVS4+191 G>A SNP at position 211 in this sequence
 ACCTGAGATA GTGGGAAAAG gtaagaat~~t~~ a~~t~~att~~g~~acag taaaaagg~~t~~ct tctaaaatgt 60
 50 atacattt~~a~~ ttacatct~~c~~ aaaaattgtt gtgat~~t~~tca tt~~g~~cca~~a~~at ttaattaaga 120
 at~~g~~aaat~~g~~ga a~~a~~acat~~t~~tg actcttacag acataattat agt~~g~~ttaata tacacag~~t~~c 180
 gcccattt~~a~~ac a~~a~~cacag~~g~~tt taaactac~~G~~c Gttt~~t~~cactt ctat~~g~~caat~~t~~tt~~g~~tccatc 240
 tgaactggat gataaac~~c~~t~~g~~ ccggtaagaa tat~~c~~tgacat tttctatatt tggatt~~g~~aac 300

Sequence ID No 8

IVS5-118 delCTTGTA deletion of 6bp from position 63 to 68 incl.

5 tagcagcata agaatggact aatacaccat attgtcaaag tttgcaaaagt gaatataaat 60
taCTTGTAct tgtaaattaa aaaaaataa gtagaataat taagagttt caagtagtt 120
aattttaat agaaaatgctt aaattaatgt ttaaaatgaa acactctttt atctacatag 180
GTTGTTAAA GGAATCTGGG 200

10

Sequence ID No 9 IVS6+33 C>T SNP at position 53 in this sequence
TATTGGATAT GTAGATCTAA gtaagtacaa ccagaacaag gtaccatgtt aaCgttttc 60
taagcacaca tgcggaaaac atttttcaa ataactgaat tcactcttc aatagtcctt 120
tgcttaatat aatttagaaag ttacaagtag gaaataatg tattactaat cagaataat 180
ataaaaatcca gctccatattt 200

15

Sequence ID No 10

IVS10-107 Ins TTC SNP at position 75 in this sequence

20 taaaaaaaaa ctttgcatt tcgtcatcat caaagcaaattt ttcttcataat aaagaaaaat 60
tcttttatcta cttt(TTC)tttcc ctctttctct gctttcactt tacttcttcc ttcttcctccc 120
cttctttgtc ttttttctct ctctctcttct ttttgatata tgtctatcat atatttccag 180
AAATAATCCA GTGACATCTC 200

25

Sequence ID No 11

IVS11+142 InsT InsT at position 162 in this sequence

CATGTCATGC TGATTGTTAA gtaagtatgaa cttttaaaaa cattttcata tgcatacgac 60
tataaacaca ccttaatgata tgcataatttt tacataatata actggaaattt caaattcata 120
tttcatcaaa tttaatttt ctgagaatttcc attttattaa aa(T)ttactatg aactctcaag 180
gctgtatata ataattttgc 200

35

Sequence ID No 12

IVS13-97 G>C SNP at position 84 in this sequence

tgtatgggt ctttgagatt tctaataatc tttattattt ggttagatgca gaacaaaata 60
ataaaacgaat cctccaaattt tttGaaacttt tatataatca aaatataatca atgtggaaata 120
tcatgcaggat acataaaaa tatgtttccctt aaactgacat cttctttctt cctattacag 180
GAGGAATTCT AGCTCCAATA 200

OATPC promoter region

45 [0069] Total length of the sequence = 1538bp
1500bp of OATPC sequence directly upstream from the cDNA sequence
Sequence in uppercase represents 38bp overlap with the cDNA sequence (SEQ ID NO 1) where this 38bp is 5'UTR sequence.
Nucleotide positions in the promoter have been determined where the -1 position is the base (lowercase) directly upstream of the end of the cDNA sequence.

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	atgcttttg acctctgaaa atattggaga attttaaac	tggcacccttt agctcaggat	60
	tataaagggtt gttagtttagt ttgtactgtt ttatcttcat	tgtatataat atatatatta	120
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	ggagtgtact cttaaaaactt acttgggtttt atatgtttgt	ctagagcaat gacataat	1440
	ggtggttaat catctatggaa ctgtttttca aaaaaggccac	tactttttttt ggaataaaagg	1500
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SEQUENCE LISTING

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 35 40 45
 Lys Ser Ser Ile Ile His Ile Glu Arg Arg Phe Glu Ile Ser Ser Ser
 15 50 55 60
 Leu Val Gly Phe Ile Asp Gly Ser Phe Glu Ile Gly Asn Leu Leu Val
 65 70 75 80
 Ile Val Phe Val Ser Tyr Phe Gly Ser Lys Leu His Arg Pro Lys Leu
 20 85 90 95
 Ile Gly Ile Gly Cys Phe Ile Met Gly Ile Gly Gly Val Leu Thr Ala
 100 105 110
 Leu Pro His Phe Phe Met Gly Tyr Tyr Arg Tyr Ser Lys Glu Thr Asn
 25 115 120 125
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 130 135 140
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 30 145 150 155 160
 Gly Cys Leu Lys Glu Ser Gly Ser Tyr Met Trp Ile Tyr Val Phe Met
 165 170 175
 Gly Asn Met Leu Arg Gly Ile Gly Glu Thr Pro Ile Val Pro Leu Gly
 35 180 185 190
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 195 200 205
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 40 Thr Leu Gly Ser Leu Phe Ser Lys Met Tyr Val Asp Ile Gly Tyr Val
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 45 Ala Trp Trp Leu Asn Phe Leu Val Ser Gly Leu Phe Ser Ile Ile Ser
 260 265 270
 Ser Ile Pro Phe Phe Phe Leu Pro Gln Thr Pro Asn Lys Pro Gln Lys
 275 280 285
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 290 295 300
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 55 Asn Val Thr Gly Phe Phe Gln Ser Phe Lys Ser Ile Leu Thr Asn Pro
 325 330 335

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	Leu Tyr Val Met Phe Val Leu Leu Thr Leu Leu Gln Val Ser Ser Tyr			
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5	Ile Gly Ala Phe Thr Tyr Val Phe Lys Tyr Val Glu Gln Gln Tyr Gly			
	355	360	365	
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	405	410	415	
15	Met Ser Leu Ser Phe Tyr Leu Leu Tyr Phe Phe Ile Leu Cys Glu Asn			
	420	425	430	
	Lys Ser Val Ala Gly Leu Thr Met Thr Tyr Asp Gly Asn Asn Pro Val			
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20	Thr Ser His Arg Asp Val Pro Leu Ser Tyr Cys Asn Ser Asp Cys Asn			
	450	455	460	
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	Lys Pro Ile Val Phe Tyr Asn Cys Ser Cys Leu Glu Val Thr Gly Leu			
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30	Gln Asn Arg Asn Tyr Ser Ala His Leu Gly Glu Cys Pro Arg Asp Asp			
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	Ala Cys Thr Arg Lys Phe Tyr Phe Phe Val Ala Ile Gln Val Leu Asn			
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40	Met Val Ile Arg Ala Leu Gly Gly Ile Leu Ala Pro Ile Tyr Phe Gly			
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Claims

1. A method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

20 positions 510, 696, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;

25 positions 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;

25 positions 321 and 1332 defined by position in SEQ ID NO 3;

30 position 41 defined by position in SEQ ID NO 4;

30 positions 109 and 244 defined by position in SEQ ID NO 5;

30 positions 117 and 283 defined by position in SEQ ID NO 6;

30 positions 209 and 211 defined by position in SEQ ID NO 7;

30 positions 63 to 68 defined by position in SEQ ID NO 8;

30 position 53 defined by position in SEQ ID NO 9;

30 position 75 defined by position in SEQ ID NO 10;

30 position 162 defined by position in SEQ ID NO 11; and

35 positions 84 defined by position in SEQ ID NO 12.

2. Use of a method as defined in claim 1 to assess the pharmaco genetics of a drug transportable by OATPC.

35 3. A polynucleotide comprising at least 20 bases of the human OATPC gene and comprising an allelic variant selected from any one of the following:

Region	variant	Position in SEQ ID NO	SEQ ID NO
Exon 4	A	510	1
Exon 5	T	670	1
Exon 5	T	696	1
Exon 9	G	1299	1
Exon 9	A	1312	1
Exon 9	A	1347	1
Exon 10	C	1561	1
Exon 14	C	2028	1
3'UTR	Insert T	2327	1
3'UTR	C	2342	1
Promoter	G	321	3
Promoter	C	1332	3
Intron 1	A	41	4

(continued)

Region	variant	Position in SEQ ID NO	SEQ ID NO
Intron 2	G	109	5
Intron 2	G	244	5
Intron 3	A	117	6
Intron 3	A	283	6
Intron 4	A	209	7
Intron 4	A	211	7
Intron 4	Deletion CTTGTA	63	8
Intron 6	T	53	9
Intron 9	Insert TTC	75	10
Intron 11	Insert T	162	11
Intron 12	C	84	12

- 20 4. A nucleotide primer which can detect a polymorphism as defined in claim 1.
- 5 5. An allele specific primer capable of detecting a OATPC gene polymorphism as defined in claim 1.
- 10 6. An allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism as defined in claim 1.
- 15 7. Use of an OATPC polymorphism as defined in claim 1 as a genetic marker in a linkage study.
- 20 8. Use of a drug transportable by OATPC in preparation of a medicament for treating a disease in a human detected as having a OATPC polymorphism at one or more of the following positions:
- 25 positions 487, 510, 554, 670, 696, 819, 820, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
- 30 positions 130, 152, 174, 241, 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
- 35 positions 321 and 1332 defined by position in SEQ ID NO 3;
- 40 position 41 defined by position in SEQ ID NO 4;
- 45 positions 109 and 244 defined by position in SEQ ID NO 5;
- 50 positions 117 and 283 defined by position in SEQ ID NO 6;
- 55 positions 209 and 211 defined by position in SEQ ID NO 7;
- 60 positions 63 to 68 defined by position in SEQ ID NO 8;
- 65 position 53 defined by position in SEQ ID NO 9;
- 70 position 75 defined by position in SEQ ID NO 10;
- 75 position 162 defined by position in SEQ ID NO 11; and
- 80 positions 84 defined by position in SEQ ID NO 12.
- 85 9. A use according to claim 10 in which the drug is a statin.
- 90 10. A use according to claim 10 in which the drug is rosuvastatin.
- 95 11. An allelic variant of human OATPC polypeptide comprising at least one of the following:
- 100 a leucine at position 400 of SEQ ID NO 2;
- 105 an isoleucine at position 405 of SEQ ID NO 2;
- 110 an arginine at position 488 of SEQ ID NO 2;
- 115 a phenylalanine at position 643 of SEQ ID NO 2;
- 120 or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic

variant.

12. An antibody specific for an allelic variant of human OATPC polypeptide as defined in claim 11.

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Europäisches Patentamt

European Patent Office

Office européen des brevets



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EUROPEAN PATENT APPLICATION

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(54) Polymorphisms in the human organic anion transporter C (OATP-C) gene

(57) This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic var-

iation in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs such as statins.

EP 1 186 672 A3



European Patent
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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 01 30 6983
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X,D	WO 00 08157 A (HU PING ;LAUBERT BORIS (US); AXYS PHARM INC (US); BUCKLER ALAN J () 17 February 2000 (2000-02-17) * page 6, line 18 - page 8, line 27 * * page 11, line 18 - page 16, line 32 * * example 1 * * claims 1-5,10,15,17 *	1-3,5-7	C12Q1/68 C07K14/47 C07K16/18
A	---	11,12 -/-	
			TECHNICAL FIELDS SEARCHED (Int.Cl.)
			C12Q C07K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search: see sheet C</p>			
Place of search	Date of completion of the search	Examine:	
MUNICH	15 July 2002	Ulbrecht, M	
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons S : member of the same patent family, corresponding document</p>			



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INCOMPLETE SEARCH
SHEET C

Application Number
EP 01 30 6983

Claim(s) searched incompletely:
5

Claim(s) not searched:
4

Reason for the limitation of the search:

- 1) As essentially any primer hybridising to the chromosome harbouring the OATPC gene is suitable for the detection of the claimed OATPC polymorphisms and as furthermore the length of said primer is subject to almost infinite variation, the subject-matter of claim 4 lacks clarity within the meaning of Article 84 to an extent as to render a meaningful search of said claim impossible.
- 2) The term allele specific primer used in claim 5 is not clear causing problems in construing the scope of said claim (Article 84 EPC). Based on the description of the present application the search for such a primer was limited to a primer applicable to ARMS methodology in which the polymorphic position is located at the 3' end of said primer.

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PARTIAL EUROPEAN SEARCH REPORT

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EP 01 30 6983

Category	Citation of document with indication, where appropriate, of relevant passages	CLASSIFICATION OF THE APPLICATION (Int.Cl.)	
		Relevant to claim	TECHNICAL FIELDS SEARCHED (Int.Cl.)
X,D	<p>TAMAI I ET AL: "MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF NOVEL MEMBERS OF THE HUMAN ORGANIC ANION TRANSPORTER (OATP) FAMILY" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 273, no. 1, 2000, pages 251-260, XP000941538 ISSN: 0006-3002 * page 252, left-hand column, paragraph 4 - right-hand column, paragraph 3 * * page 253, right-hand column, paragraph 2 - page 254, right-hand column, line 1 * * figures 1,3,4 *</p>	1-3,5-7	
A	<p>& DATABASE EMBL [Online] EBI; 8 June 1999 (1999-06-08) TAMAI I ET AL.: "Homo sapiens mRNA for organic anion transporter OATP-C" retrieved from HTTP://WWW.EBI.AC.UK/CGI-BIN/EMBLFETCH Database accession no. AB026257 * the whole document *</p>	11,12 1-3,5-7, 11,12	
A	<p>DATABASE EMBL [Online] EBI; 2 February 2000 (2000-02-02) MUZNY DM ET AL.: "Homo sapiens 12p BAC RP11-12505" retrieved from HTTP://WWW.EBI.AC.UK/CGI-BIN/EMBLFETCH Database accession no. AC022335 XP002188259 * the whole document *</p>	1-3,5-7	
		-/-	

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Application Number

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.)
			TECHNICAL FIELDS SEARCHED (Int.Cl.)
A,D	<p>HSIANG B ET AL: "A NOVEL HUMAN HEPATIC ORGANIC ANION TRANSPORTING POLYPEPTIDE (OATP2)" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 52, 1999, pages 37161-37168, XP000877290 ISSN: 0021-9258 * abstract * * page 37164, right-hand column, paragraph 2 * * figures 3,4 *</p> <p>---</p>	1-3,5-7, 11,12	
A	<p>ULBRECHT M ET AL.: "Assoziation of beta 2-adrenoreceptor variants with bronchial hyperresponsiveness" AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, vol. 161, no. 2, February 2000 (2000-02), pages 469-474, XP002188594 * page 470, left-hand column, paragraph 5 - right-hand column, paragraph 2 *</p> <p>---</p>	1	
A	<p>WO 99 06046 A (KOS PHARMA INC) 11 February 1999 (1999-02-11) * abstract * * claims 1,3 *</p> <p>-----</p>	2	

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 30 6983

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
 The members are as contained in the European Patent Office EDP file on
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15-07-2002

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			EP	1141282 A2	10-10-2001
			WO	0008157 A2	17-02-2000
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			AU	8680098 A	22-02-1999
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			WO	9906046 A1	11-02-1999

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